New triterpenoids from the leaves of *Photinia serrulata*

Li-Bing Yang[a,b] and Li-Xing Zhao[a]*

[a]*The Key Laboratory for Microbial Resources of the Ministry of Education, Laboratory for Conservation and Utilisation of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, P.R. China*

[b]*Department of Pharmacy, Xi'an Medical University, Xi'an 710021, P.R. China*

A new triterpenoid, 3β,15α-dihydroxy-12-keto-9(11)-ursene, and three known analogues, were isolated from the leaves of *Photinia serrulata*. The structure of the new compound, including its relative configuration, was elucidated by spectroscopic analysis, especially by two dimensional NMR techniques.

Keywords: *Photinia serrulata*, triterpene, rosaceae, ku-ding-cha

Photinia serrulata (the family Rosaceae) is distributed throughout the East and South of China. Its young leaves are used as vegetables, and the mature leaves are used as 'ku-ding-cha' in some regions of China. Ku-ding-cha is generally consumed in southern China as a type of tea. The beverage is light green and the taste is very bitter giving rise to the Chinese name, ku-ding-cha, which means a tea having bitter taste.[1,2] This tea is also used as a herbal medicine to treat arteriosclerosis and obesity.[3]

Previous phytochemical analysis has revealed that the leaf essential oil of *P. serrulata* exhibited cytotoxicity towards human cancer cell lines and possessed antioxidant activity.[4] With the aim of isolating bioactive natural products from Chinese Medicine, we investigated the chemical constituents of *P. serrulata*. A new ursane triterpene (**1**), three known analogues, 3β-hydroxy-12-keto-9(11)-ursen-28,13β-olide (**2**),[5,6] tormentic acid (**3**),[7] and ursolic acid (**4**), were isolated. The isolation and structure elucidation of triterpenoid are reported in this paper.

Compound **1** was obtained as amorphous powder with molecular formula $C_{30}H_{48}O_3$ which was determined by HRESIMS. The IR absorption at 3502, 1689 and 1642 cm^{-1}, indicated the presence of hydroxyl groups, carbonyl group, and double bond. ^1H NMR spectrum contained six tertiary methyl signals, two secondary methyls, one olefinic signal (δ_H 6.25, s) and two oxymethine signals at δ_H 4.54 and 3.49 (Table 1). The ^{13}C NMR and DEPT spectra revealed a total of 30 carbon signals including eight methyls, seven methylenes, six methines including two oxygenated ones, two olefinic carbons (δ_C 180.2 and 123.5), one α,β-unsaturated carbonyl (δ_C 201.1), and five quaternary carbons (Table 1). These NMR data together with the molecular formula suggested that **1** has five rings. Considering the structures of the known triterpenoids isolated from this plant, along with the characteristic two doublet methyl signals at δ_H 1.03 (d, J = 6.5 Hz) and 0.82 (d, J = 6.5 Hz), respectively, and five unoxygenated quaternary carbon signals at δ_C 39.9 (s), 47.3 (s), 40.7 (s), 47.1 (s), and 35.7 (s) due to C-4, 8, 10, 14 and 17, respectively. Compound **1** was tentatively assigned an ursane skeleton. This was confirmed by examination of 2D NMR data. The two oxymethines were ascribed to C-3 and C-15, respectively, on the basis of HMBC correlations of H_3-27 and H_3-28 with C-3, and of H_3-24 with C-8, C-13, C-14, and C-15. The presence of a α,β-unsaturated ketone at C-12 was determined by the couplings of H-11, H-13, and H-18 with C-12 in the HMBC spectrum.

The relative stereochemistry of the molecule was deduced from coupling constants (Table 1) and correlations observed in ROESY spectrum (Fig. 2). The β-orientation for the C-3 OH was suggested from the intense ROESY correlations of H-3 with H-1α, H-5, and H_3-28. The *cis* coupling constant between H-13 and H-18 (J = 3.6 Hz) and ROESY cross-peaks of H-13 with H_3-23 and H_3-25 indicated the β-orientation of H-13 and H-18 in **1**. Additionnally, the ROEs from H-15 to H-13, H_3-23 and H_3-25 indicated the β-orientation of H-15. Therefore, compound **1** was elucidated as 3β,15α-dihydroxy-12-keto-9(11)-ursene.

Compounds **1–3** were tested for cytotoxicity against A549 cells using the sulforhodamine B (SRB) method. However, they were completely inactive with IC_{50} values of >100 μM.

Table 1 ^1H and ^{13}C NMR data of **1** at 500 MHz in pydrine-d_5[a]

No.	δ_H J (Hz)	δ_C	No.	δ_H J (Hz)	δ_C
1α	1.30 (m)	37.1 (t)	16α	2.28 (t, 12.0)	39.8 (t)
1β	1.96 (m)		16β	1.47 (dd, 12.0, 4.8)	
2	1.86-1.94 (2H, m)	28.5 (t)	17		35.7 (s)
3	3.49 (dd, 12.0, 3.2)	77.2 (d)	18	2.38 (dd, 11.2, 3.6)	47.9 (d)
4		39.9 (s)	19	1.70 (m)	40.0 (d)
5	1.12 (brd, 11.2)	50.7 (d)	20	1.07 (overlapped)	39.7 (d)
6α	1.58 (m)	18.6 (t)	21α	1.36 (m)	31.9 (t)
6β	1.73 (m)		21β	1.24 (m)	
7α	2.54 (m)	36.9 (t)	22	1.38 (2H, m)	41.7 (t)
7β	2.03 (m)		23	1.05 (s)	29.5 (q)
8		47.3 (s)	24	1.49 (s)	14.6 (q)
9		180.2 (s)	25	1.38 (s)	25.3 (q)
10		40.7 (s)	26	1.18 (s)	24.9 (q)
11	6.25 (s)	123.5 (d)	27	1.04 (s)	16.6 (q)
12		201.1 (s)	28	1.22 (s)	28.8 (q)
13	3.05 (d, 3.6)	49.0 (d)	29	1.03 (s)	20.8 (q)
14		47.1 (s)	30	0.82 (s)	21.2 (q)
15	4.54 (dd, 12.0, 4.8)	67.0 (d)			

[a]The assignments were based on ^1H–^1H COSY, HMQC, HMBC, and ROESY experiments.

* Correspondent. E-mail: lixing.zhao@yahoo.com

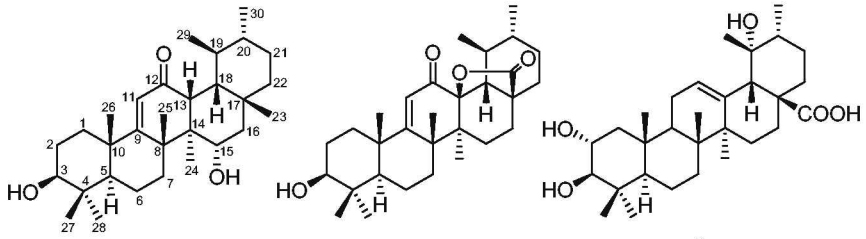

Fig. 1 Structures of compounds **1–3**.

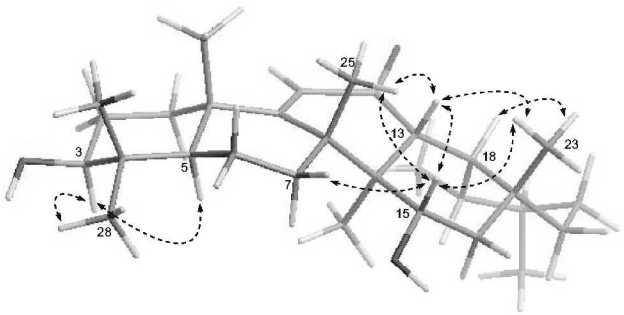

Fig. 2 Key ROESY correlations of **1**.

Experimental

IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. Optical rotations were determined on a Perkin–Elmer model 241 polarimeter. MS were recorded on a VG Auto spec-3000 spectrometer. 1D and 2D NMR spectra were measured on a Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed with silica gel 60 (200–300 mesh, Merck), Sephadex LH-20 (Pharmacia), and reversed-phase C-18 silica gel (250 mesh, Merck). Pre-coated TLC sheets of silica gel 60 GF254 were used. Agilent 1100 series HPLC equipped with an Alltima C-18 column (4.6 × 250 mm) was used for HPLC analysis and a semipreparative Alltima C-18 columns (9.4 × 250 mm) were used in sample preparation.

Plant material

The leaves of *P. serruiata* were collected in Qinglin mountain, Shanxi Province, China and were identified by Jing Ling. Voucher specimens (CNY 060920) were deposited in Xi'an Medical University.

Extraction and isolation

The air-dried leaves (3.1 kg) of *P. Serruiata* were extracted with 95% ethanol (10 l × 3, each 2 days) at room temperature. The filtrate was evaporated, and the resulting residue was partitioned between water and ethyl acetate. The ethyl acetate fraction (110 g of dry extract) was purified by column chromatography (1 kg of silica gel), chloroform–acetone from 1:0 to 0:1), affording fractions A–F. After repeated column chromatography (SiO_2; gradient mixtures of chloroform–methanol), fraction B afforded compound **4** (280 mg) and subfraction B4 (790 mg). Subfractions B4 was subjected to Sephadex LH-20 column eluted with methanol to yield 3 fractions, B41-B43. B41 was finally purified by semipreparative HPLC to give compounds **1** (6.5 mg) and **2** (5.4 mg). Compound **3** was obtained from fraction C by RP-18 column chromatography eluted in a step gradient manner with methanol/water (from 40:60 to 100:0).

3β,15α-dihydroxy-12-keto-9(11)-ursene (**1**): $[\alpha]^{23}_D$ + 15.9 (c 0.20, MeOH); IR (KBr) ν_{max} 3502, 2945, 2870, 2854, 1689, 1642, 1592, 1466, 1377, 1046, 960 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Table 1; HRESIMS (positive ion) *m/z* 455.3524 (calcd for $C_{30}H_{49}O_3$ $[M + H]^+$, 455.3525).

Received 25 July 2008; accepted 15 August 2008
Paper 08/0070 *doi: 10.3184/030823408X360337*
Published online: 10 November 2008

References

1 K.M. Lau, Z.D. He, H. Dong, K.P. Fung and P.P.H. But, *J. Ethnopharmacol.*, 2002, **83**, 63.
2 O. Negishi, Y. Negishi, F. Yamaguchi and T. Sugahara, *J. Agric. Food Chem.*, 2004, **52**, 5513.
3 K. Nishimura, T. Fukuda, T. Miyase, H. Noguchi and X.M.A. Chen, *J. Nat. Prod.*, 1999, **62**, 1061.
4 J. Hou, T. Sun, J. Hu, S.Y. Chen, X.H. Cai and G.L. Zou, *Food Chem.*, 2007, **103**, 355.
5 Y.L. Song, Y.H. Wang, Q. Lu, J. Gao, M. Bi and Y.X. Cheng, *Molecules*, 2007, **12**, 2599.
6 Y.L. Song, Y.H. Wang, Q. Lu, H.J. Qiao and Y.X. Cheng, *Helv. Chim. Acta*, 2008, **91**, 665.
7 A. Villar, M. Payá, M.D. Hortigüela and D. Cortes, *Planta Med.*, 1986, **52**, 43.
8 A. Numata, P.M. Yang, C. Takahashi, R. Fujiki, M. Nabae and E. Fujita, *Chem. Pharm. Bull.*, 1989, **37**, 648.